RESVERATROL PLAYS A PROTECTIVE ROLE IN THE LIVER BY REGULATING APOPTOSIS AND SIRTI/HMGB1 SIGNALING PATHWAYS

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ABSTRACT

Objective*: To investigate the protective effect of resveratrol (Res) on the liver by regulating the signaling pathway of apoptosis and silencing regulator 1 (SIRT1)/high-mobility group protein B1 (HMGB1).*

Methods: *6-week-old male SD rats (48) were randomly divided into Sham, Hepatectomy and Res treatment groups, with 16 rats in each group. In the Sham group, simple abdominal dissection and extrahepatic vascular separation were performed, and 1mL·kg-1, 1d/1 of normal saline was intraperitoneally injected at 1w before surgery, until the end point of observation. Rats in the Hepatectomy group underwent partial hepatectomy, and the remaining procedures were the same as those in the Sham group. All rats in the Res group underwent partial hepatectomy, and Res 30mg·kg-1 was intraperitoneally injected at the same time at 1w before surgery, for 1d/time, until the end point of observation. The apoptosis number, acetylated HMGB1 and SIRT1 protein expression levels, serum HMGB1 levels and serum HMGB1 levels of rats in each group were compared after hepatectomy.*

Results: *The number of apoptosis of hepatocytes in the Hepatectomy group was significantly higher than that in the Sham group (P<0.05). Moreover, the number of apoptosis of hepatocytes in the Res group was significantly lower than that in the Hepatectomy group (P<0.05). At 30min, 24, 48 and 72h, the HMGB1 level of rats in the Hepatectomy group was significantly higher than that in the Sham group (P<0.05). The HMGB1 levels of rats in the Res group were significantly lower than those in the Sham group (P<0.05). The expression level of acetylated HMGB1 protein in the rat liver tissues of the Hepatectomy group was significantly higher than that of the Sham group, and the expression level of SIRT1 protein was significantly lower than that of the Sham group. However, the expression level of acetylated HMGB1 protein in the liver tissue of rats in the Res group was significantly lower than that in the Hepatectomy group, and the expression level of SIRT1 protein was significantly higher than that in the Hepatectomy group (P<0.05).*

Conclusion: *Resveratrol may play a protective role in the liver by inhibiting apoptosis, possibly through the SIRTI/HMGB1 signaling pathway.*

Keywords: *Hepatocellular carcinoma, resveratrol, apoptosis, SIRTI, HMGB1.*

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Introduction

Hepatocellular carcinoma is one of the most difficult malignant diseases to cure at present. Hepatectomy is currently the most effective method to treat hepatocellular carcinoma in the early stage of onset, which can significantly prolong patients' survival time[1]. However, risk of liver failure is significantly increased after subtotal hepatectomy, which seriously threatens the life and health of patients. In addition, due to the liver's abundant blood supply, the difficulty of the operation itself is significantly increased; in particular, the hepatic hilum blocking treatment can lead to ischemia-reperfusion injury, which eventually leads to dysfunction of organs such as the heart, liver and kidneys[2]. Therefore, it is urgent to find a new and effective treatment method to prevent and treat postoperative liver failure. Resveratrol (Res) is extracted from grape plants and was first discovered in 1997. Some scholars have found that Resveratrol can reduce the incidence of skin cancer in mouse models, and since then, more and more scholars have paid increasing attention to Resveratrol[3].

Many studies have confirmed that Resveratrol has extensive biological activity that can inhibit the abnormal apoptosis of cells and inhibit the immune inflammatory response, and also has significant tissue protection function within organs such as the heart, liver and kidneys. Apoptosis is a negative regulatory mechanism that plays a crucial role in the process of liver regeneration after hepatectomy. A certain degree of apoptosis can promote the elimination of damaged liver cells, which is conducive to the recovery of liver function[4]. High mobility group protein B1 (HMGB1) is a non-histone chromosomal binding protein mainly existing in the nucleus, which can bind to DNA-specific sites and further regulate DNA replication, repair, recombination and transcription processes, thus contributing to the maintenance of nucleosomal stability[5].

Previous studies have confirmed that HMGB1 plays a key role in the occurrence and development of many liver diseases as an inflammatory mediator[6]. As a deacetylase, silent mating type information regulation 2 homolog 1 (SIRT1) can praise the gene transcription and cell aging processes of the body. Relevant studies on liver cancer have shown that SIRT1's expression in cancer tissues is increased compared with that in normal liver tissues, possibly because SIRT1 has an important regulatory function in the origin and development of liver cancer[7]. Based on the above studies, this study intends to analyze the protective effect of Resveratrol on liver through the regulation of cell apoptosis and the SIRTI/HMGB1 signaling pathway as well as its mechanism.

Materials and methods

Experimental animals

6-week-old male SD rats (48) were purchased from Reed Liver Disease Research (Shanghai) Co., LTD., weighing 210-230g. A total of 48 rats were kept in a clean animal house at a temperature of 25°C, and their circadian rhythm was replaced once every 12 hours so that they could live a normal life. The rats were allowed to eat and drink freely. This study protocol conforms to the regulations on the use and management of experimental animals.

Main reagents and instruments

Reagent

4% paraformaldehyde was purchased from Beijing Regen Biotechnology Co., LTD. Resveratrol was purchased from Nanjing Dosf Biotechnology Co., LTD. TUNEL in situ apoptosis detection kit was purchased from JianJian Institute of Biological Engineering, Nanjing. BCA protein quantitative kit was purchased from Beijing Dingguo Changsheng Biotechnology co., LTD. Acetylated HMGB1, HMGB1, SIRT1, β-actin rabbit anti-human polyclonal antibodies were purchased from Beijing Kangruncheng Biotechnology Co., LTD.

Instrument

-80 °C ultra-low-temperature refrigerator was purchased from Qingdao Haier Bio-medical Co., LTD. High-speed centrifuge was purchased from Beijing Taizejia Technology Development co., LTD. Super-clean workbench was purchased from Shanghai Chenlian Biotechnology Development Co., LTD. Inverted fluorescnec microscope was purchased from Guangzhou Mingmei Optoelectronic Technology Co., LTD.

Methods

• 48 SD rats were randomly divided into Sham, Hepatectomy and Res treatment groups, with 16 rats in each group. In the Sham group, simple abdominal dissection and extrahepatic vascular separation were performed, and 1mL·kg-1, 1d/1 of normal saline was intraperitoneally injected at 1w before surgery, until the end point of observation. Rats in the Hepatectomy group underwent partial hepatectomy, and the remaining procedures were the same as those in the Sham group. All rats in the Res group underwent partial hepatectomy, and Res 30mg·kg-1 was intraperitoneally injected at the same time at 1w before surgery, for 1d/ time, until the end point of observation.

• The observation time points were set for 30min, 24h, 48h, and 72h. At the observation point, the rats were treated and their abdominal cavities were exposed. 1mL of inferior vena cava blood was taken and centrifuged for 10min under the condition of 4000r/min to obtain serum 250~350 living L.

• Liver tissues such as the right lobe and caudate lobe of the rat liver were weighed, part of the tissues were soaked in 4% formaldehyde solution for 48h, and the remaining tissues were placed in a frozen storage tube and stored in liquid nitrogen for detection.

Observation indicators

• TUNEL was used to measure the apoptosis of hepatocytes in SD rats after hepatectomy.

• Serum HMGB1 levels of rats after hepatectomy

in each group were determined by ELISA at 30min, 24h, 48h and 72h.

• Western blot was used to determine the expression levels of acetylated HMGB1 and SIRT1 proteins in the liver tissues of rats after hepatectomy.

Statistical methods

The number and HMGB1 level of hepatocyte apoptosis after hepatectomy were measured by (x±s). The two groups were compared by independent sample t test. One-way analysis of variance was used for comparison between groups, and LSD-t test was used for comparison between groups. All study data were analyzed and processed with SPSS23.0, and P<0.05 was considered statistically significant.

Results

Comparison of hepatocyte apoptosis after hepatectomy in each group

The quantity of apoptosis of hepatocytes in the Hepatectomy group was significantly higher than that in the Sham group (P<0.05). Moreover, the quantity of apoptosis of hepatocytes in the Res group was significantly lower than that in the Hepatectomy group (P<0.05). See Table 1, Figure 1.

Group	Quantity of apoptosis
Sham group	49.11±6.71
Hepatectomy group	150.82±14.61*
Res group	93.14±10.02#

Table 1: Quantity of hepatocyte apoptosis after hepatectomy in each group ($\bar{x}$±s).
*Note: *indicates that compared with Sham group, P<0.05; #indicates compared with the Res treatment group, P<0.05.*

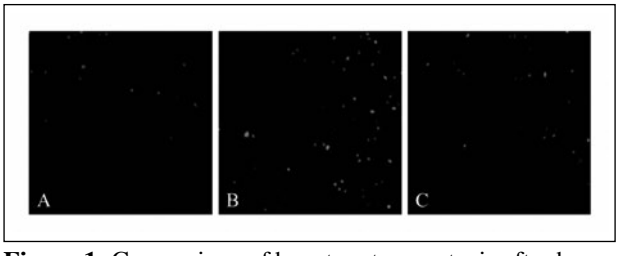

Figure 1: Comparison of hepatocyte apoptosis after hepatectomy in each group.
Note: figure A: Sham group; Figure B: Hepatectomy group; Figure C: the Res processing group.

Comparison of serum HMGB1 levels after hepatectomy in each group

At 30min, 24, 48 and 72h, the HMGB1 level of rats in the Hepatectomy group was significantly higher than that in the Sham group (P<0.05). The HMGB1 levels of rats in the Res group were significantly lower than those in the Sham group (P<0.05), as shown in Table 2.

Group	30min (ng/mL)	24h (ng/mL)	48h (ng/mL)	72 (ng/mL)
Sham group	8.41±0.63	7.61±0.22	4.62±0.52	2.71±0.30
Hepatectomy group	11.61±0.53*	9.31±0.49*	7.91±0.98*	6.01±0.75*
Res group	10.41±0.39#	8.21±0.31#	6.61±0.51#	3.79±0.29#

Table 2: Comparison of serum HMGB1 levels after hepatectomy in each group.
*Note: *indicates that compared with Sham group, P<0.05; #indicates compared with the Res treatment group, P<0.05.*

Comparison of the expression levels of acetylated HMGB1 and SIRT1 proteins in the liver tissues of rats after hepatectomy in each group

The expression level of acetylated HMGB1 protein in the rat liver tissues of the Hepatectomy group was significantly higher than that of the Sham group, and the expression level of SIRT1 protein was significantly lower than that of the Sham group. However, the expression level of acetylated HMGB1 protein in the liver tissue of rats in the Res group was significantly lower than that in the Hepatectomy group, and the expression level of SIRT1 protein was significantly higher than that in the Hepatectomy group (P<0.05). See Table 3, Figure 2.

Group	HMGB1	SIRT1
Sham group	0.13±0.04	0.38±0.05
Hepatectomy group	0.52±0.06*	0.07±0.03*
Res group	0.28±0.04[3]	0.20±0.03#

Table 3: Comparison of the expression levels of acetylated HMGB1 and SIRT1 proteins in the liver tissues of rats after hepatectomy in each group.
*Note: *indicates that compared with Sham group, P<0.05; #indicates compared with the Res treatment group, P<0.05.*

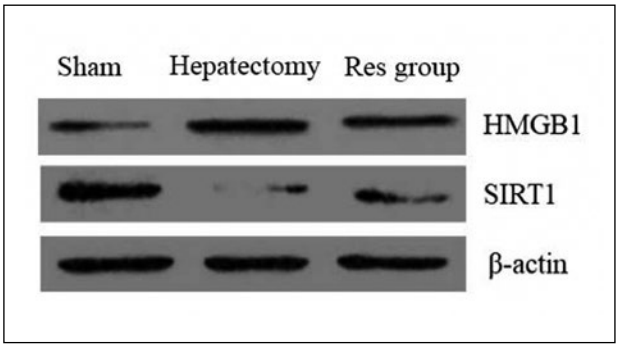

Figure 2: Comparison of HMGB1 and SIRT1 protein expression levels in liver tissues of rats after hepatectomy.

Discussion

Excessive apoptosis of hepatocytes is an important factor contributing to liver injury. Previous studies have shown that cell necrosis is the main type of hepatocyte death caused by hepatic ischemia reperfusion injury. With the continuous progress of medical technology, other studies have found that there are many positive cells in liver tissue after hepatic ischemia reperfusion injury, suggesting that apoptosis may also be an important cause of liver cell death after hepatic ischemia reperfusion injury[8]. In this study, TUNEL was used to measure the liver tissues of rats in each group, and it was found that increased apoptosis could lead to liver injury after hepatectomy in rats, while Resveratrol could reduce the number of apoptosis in the liver tissues, suggesting that it could protect the liver by inhibiting apoptosis.

It has been reported that Resveratrol may exert a protective effect on the liver through the HMGB1 signaling pathway. Under the induction of injury factors, HMGB1 can be actively released to the outside of the cell. It can not only bind to specific receptors and play its role in promoting the inflammatory response, but also activate the inflammatory cascade reaction, leading to the secretion of many cytokines and eventually causing severe damage to the surrounding tissues[9]. It has been reported that HMGB1 can bind toll-like receptors and other receptors, which may easily lead to ischemia-reperfusion injury. In addition, HMGB1 can bind toll-like receptor 4 to activate the toll-like receptor 4 signaling pathway, and toll-like receptor 4-mediated cytokine tumor necrosis factor leuplus and interleukin-6 secretion can promote cell apoptosis, eventually leading to tissue damage[10]. It has also been reported that HMGB1-toll-like receptor 4 signal axis can be highly expressed in renal tubular epithelial cells stimulated by hypoxia and reoxygenation, and can also enhance the expression level of apoptotic factors, further aggravating tissue damage[11]. The above results suggest that inhibiting HMGB1 protein secretion can relieve the inflammatory response of organ tissue or improve the therapeutic effect to a certain extent. Furthermore, acetylation modification can play an important role in the proinflammatory function of HMGB1. Relevant studies have shown that hepatic ischemia/reperfusion can significantly inhibit the activities of histone deacetylase 1, histone deacetylase 4 and histone deacetylase 5, thereby promoting the acetylation of HMGB1 protein or extracellular secretion[12]. Extracellular HMGB1 is cytotoxic and can cause damage to the body at a certain concentration, and as the serum level of HMGB1 increases, so does the risk of organ injury[13]. Zhang et al.[14] found that Resveratrol could resist the increase of HMGB1 expression. The above results are basically consistent with the results of this study. Serum HMGB1 levels in rat hepatectomy tissues were measured, and the results showed that HMGB1 levels in the liver resection group rats were significantly higher than that of the Sham group, and that the liver tissue of acetylated HMGB1 protein expression level will increase. They also showed that the prompt serum HMGB1 after liver resection of liver injury in rats can be used as a cause of important factor, and acetylated HMGB1 also is the role of the foundation. However, the expression level of acetylated HMGB1 protein in the liver tissue of rats in the Res treatment group was significantly lower than that in the Hepatectomy group, suggesting that Resveratrol can block the acetylation modification of HMGB1 protein and can also reduce the active release of HMGB1 protein by reducing the degree of acetylation, so as to finally achieve the goal of anti-inflammatory injury.

Clinical reports have shown that SIRT1 plays an important role in the process of acetylation modification of HMGB1 protein, which can maintain intracellular stability by maintaining the deacetylation state of HMGB1 protein, and the stability of both functions can affect the progression of many liver diseases. In addition, relevant studies on warm ischemia-reperfusion injury of liver transplantation have shown that SIRT1 can reduce the release of HMGB1 protein and thus exert its protective function in liver[15].

In this study, we also found that the expression level of SIRT1 protein in the liver tissue of rats in the Res treatment group was significantly higher than that in the resection group, and the protein expression level trended inversely to the expression level of acetylated HMGB1 protein, suggesting that the protective effect of Resveratrol may be closely related to the increase of SIRT3 activity.

In summary, Resveratrol may play a protective role in the liver by inhibiting apoptosis, possibly through the SIRTI/HMGB1 signaling pathway.

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